IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s) : Keefe *et al.* Examiner: Mark Staples

Serial Number: 10/729,581 Art Unit: 1637

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Title: Method for *In Vitro* Selection of 2'-Substituted Nucleic Acids

Attorney Docket No.: 23239-544 (ARC-544)
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September 29, 2008
Cambridge, Massachusetts

DECLARATION UNDER 37 C.F.R. § 1.132

I, Anthony Keefe, hereby declare as follows:

1. I am a named inventor on the above-referenced patent application. The invention relates to materials and methods for enzymatically producing pools of randomized oligonucleotides having modified nucleotides from which aptamers to a specific target can be selected. I am informed that the claimed invention relates, in a preferred embodiment, to pools of r/mGmH nucleic acids, which are oligonucleotides containing 2'-OMe A's, U's or T's, C's and G's, where up to 10% of the G's are ribonucleotides.

2. I am informed that the claims are directed to methods for identifying an aptamer that binds to a target molecule and methods for transcribing an oligonucleotide. I am also informed that independent claims 1, 101, 102 and 182, as amended, and the claims that depend therefrom, are each directed to methods that include the step of preparing a transcription reaction mixture. In addition, I am informed that the transcription reaction mixture in claims 1 and 102 includes: (i) a modified RNA polymerase that comprises at least one mutated amino acid residue as compared to the corresponding unmodified RNA polymerase, (ii) one or more 2'-OMe modified nucleotide triphosphates (2'-OMe NTPs), including at least one 2'-OMe GTP, (iii) 2'-OH guanosine triphosphate, (iv) magnesium ions, (v) manganese ions, wherein the concentration of magnesium ions is about 3 to 5 times greater than the concentration of manganese ions, and (vi) one or more double-stranded oligonucleotide transcription templates, wherein the modified RNA polymerase exhibits an increased ability to incorporate 2'-OMe NTPs as compared to the ability of the corresponding unmodified RNA polymerase to incorporate 2'-OMe NTPs. I am further

informed that the transcription reaction mixture in claims 101 and 182 is the same as in claims 1 and 102 except that it specifies in step (vi) a different oligonucleotide transcription template.

3. I am informed that the claims have also been amended to recite that the modified RNA polymerase incorporates 2'-OMe modified NTPs, including at least one 2'-OMe GTP. In addition, I am informed that the claims have been further amended by amending step e) to recite that the methods generate a ligand-enriched mixture of nucleic acids whereby aptamers comprising at least one 2'-OMe GTP are identified.

4. I am also informed that (i) Archemix Corp. has received an Office Action having a mailing date of March 31, 2008 in the above-referenced application and (ii) the Office Action rejects the pending claims under 35 U.S.C. § 103(a) as being obvious in view of Pieken *et al.* (U.S. Patent No. 5,660,985), Brieba *et al.* (Biochemistry (2000) 39:919-923) and Sousa *et al.* (U.S. Patent No. 6,107,037); or Pieken, Brieba, Sousa and Milligan *et al.* (Methods Enzymology (1989) 180:51-62).

5. I conducted experiments to determine the effect of $MnCl_2$ and $MgCl_2$ concentration on r/mGmH transcript yield in the SELEX process. In these experiments, transcription reactions were run with a constant divalent ion concentration of 8.5 mM, but with varying concentrations of $MnCl_2$ and $MgCl_2$.

6. The transcription conditions for these experiments were as follows:

200 nM ds PCR product (transcription template);
1X Tc buffer containing:
200 mM Hepes,
40 mM DTT,
2 mM spermidine, and
0.01% Triton X-100;
8.5 mM divalents ($MgCl_2$ + $MnCl_2$)
1 mM each of mATP, mCTP, mGTP, mTTP and GMP;
30 μM rGTP (trace α-^{32}P rGTP);
inorganic pyrophosphatase; and
Y639F/H784A mutant T7 RNA polymerase.

7. Separate transcription reactions were run for each concentration of $MnCl_2$ and $MgCl_2$, with one reaction having no enzyme used as a control. The reactions were loaded onto a 10% PAGE gel, electrophoresed, exposed using a phosphoroimager screen, imaged on STORM phosphoroimager, and the bands quantitated using ImageQuant software. The raw data for each reaction was calculated and the background was subtracted in order to quantitate the reaction yield. The results of these experiments are shown in Exhibit A.

8. In my view, the data in Exhibit A clearly demonstrate that specific ratios of $MgCl_2$ to $MnCl_2$ produce a high yield of transcripts that contain any 2'-OMe modified nucleotide, including at least one 2'-OMe GTP, and that are sufficiently long for effective and efficient use in the SELEX process.

9. In my view, the transcription reaction mixture and conditions recited by the amended claims produce a high yield of transcripts that contain any 2'-OMe NTP, including 2'-OMe GTP, and that are of sufficient number and are sufficiently long for effective and efficient use in the SELEX process. Standard transcription conditions are not able to generate libraries of 2'-OMe transcripts because the polymerase is either not able to accept 2'-OMe nucleotides as substrates or the transcript terminates after the 2'-OMe NTP is incorporated. As a result, standard transcription conditions are not useful in the SELEX process. It is only the specific combination of reactants in the claimed transcription reaction mixture that works to incorporate any 2'-OMe nucleotides into transcripts with sufficient yield to be used in the iterative SELEX process.

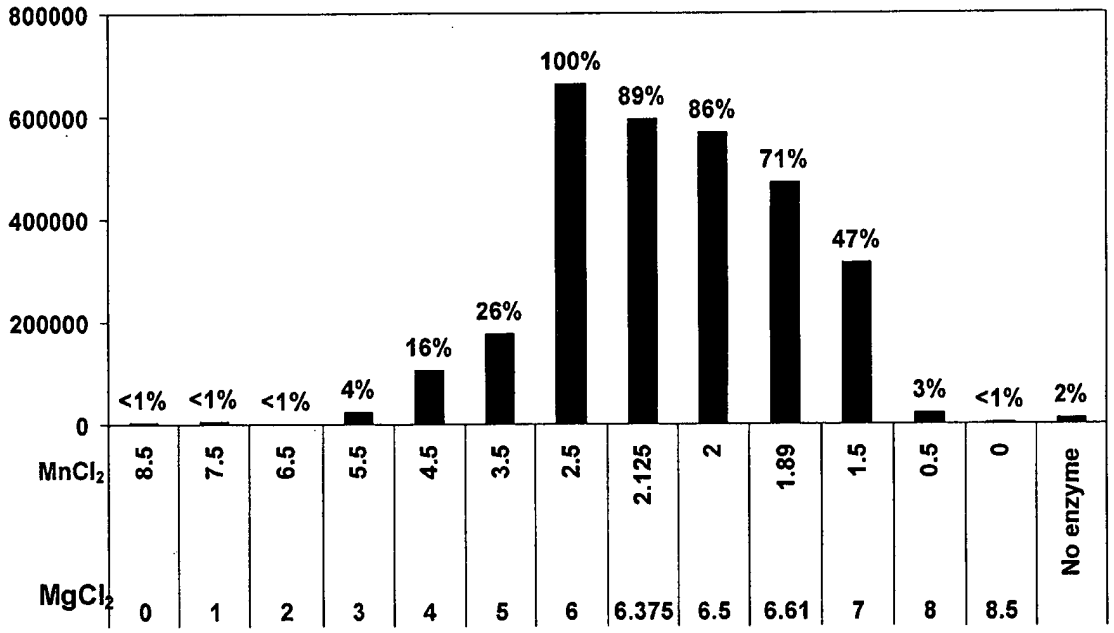
I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both under 18 U.S.C. § 1001 and that such willful false statements may jeopardize the validity of the application and any patent issued thereon.

Anthony Keefe

9/29/2008

Date

Exhibit A


800000
600000
400000
200000
0
<1%
<1%
<1%
4%
16%
26%
100%
89%
86%
71%
47%
3%
<1%
2%
MnCl2
8.5
7.5
6.5
5.5
4.5
3.5
2.5
2.125
2
1.89
1.5
0.5
0
No enzyme
MgCl2
0
1
2
3
4
5
6
6.375
6.5
6.61
7
8
8.5